Saskatchewan Agriculture, Food and Rural Revitalization

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FINAL REPORT

20000055

ULTRASONOGRAPHIC FEATURES OF THE TESTIS, EPIDIDYMIS AND ACCESSORY SEX GLANDS OF BULLS IN RELATIONSHIP TO SEMEN QUALITY AND HISTOLOGY

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Final Report

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Ultrasonographic features of the testis, epididymis and accessory glands of bulls in relationship to semen quality and histology.

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Co-investigators: Dr. Roger Pierson and Dr. Andres Arteaga

Abstract

Examination of the reproductive tissues was done on 1198 bulls at University farms in Saskatoon and Argentina, the Lethbridge Research Station, a Manitoba RPO Station, two western Canadian AI centres, Saskatchewan community pastures and mulls submitted to the Western College of Veterinary Medicine. Ultrasonography was done as early as three months of age through puberty and in mature bulls. A group of sixteen bulls was used in a scrotal insulation experiment to determine whether abnormal testis thermoregulation is involved in testis fibrosis. Semen quality in mature bulls was compared to ultrasound findings to determine whether there is a relationship between testis fibrosis and semen quality. A study was done on the phenomenon of sperm accumulation due to malfunction of the ampullae.

The results of the study indicated that fibrotic lesions appear as early as seven months of age. The incidence of fibrotic lesions within the testis parenchyma in different groups of young bulls varied from none to as high as 79.8%. It appeared the rate of growth and sexual development, low, medium, or high nutritional intake, and genetic predisposition are unlikely causes of testis fibrosis. Scrotal insulation of eight bulls for ten days did not result in fibrosis. There is some evidence that testis fibrosis may be caused by the Bovine Respiratory Syncytial Virus.

The percentage of bulls with poor semen quality tended to be higher for bulls with severe fibrosis, but even bulls with very severe fibrosis were capable of producing normal semen. An in depth investigation was done on the phenomenon of accumulation of senescent sperm. Dilatation of the ampullae visable with ultrasonography indicated that the site of accumulation of senescent sperm is primarily the ampullae. The cause of the condition appears to impaired sperm transport through the epididymis and ampullae. Affected bulls have extremely low first service conception rates during the first ten days of breeding until senescent sperm are "flushed out" and replaced by viable sperm. Senescent sperm re-accumulate within about thirty days of sexual rest.

Executive Summary

The objective of this research was to characterize the normal and abnormal ultrasonographic features of the bull reproductive tract, to determine the prevalence of reproductive tract lesions, to correlate ultrasonographic information with semen quality traits, and to use ultrasonography in conjunction with physical findings and semen information to evaluate bull fertility.

A surprisingly high prevalence of bulls with fibrotic lesions in the testis was discovered and testis fibrosis became the major focus of this study. Ultrasonography was used for the first time to demonstrate visible dilation of the ampullae in the phenomenon of sperm accumulation. A study of ampullary sperm accumulation, ampullary depletion and reaccumulation of senescent sperm and breeding trials using bulls with sperm accumulation was done.

A 5-point scale was developed for scoring testis fibrosis (Table 1) and examples of fibrosis are shown in the following figures..

Table 1. Classification method for testis fibrotic lesions.

	Number of fibrotic lesions		
Category	Small	Medium	Large
0. Normal	0	0	0
1. Slight	1-5	1-2	0
2. Mild	6-10	3-5	1-2
3. Moderate	11-20	6-10	3-5
4. Severe	21-30	11-15	6-10
5. Very Severe	>30	>15	>10

The normal features of an ultrasonogram of the testes.

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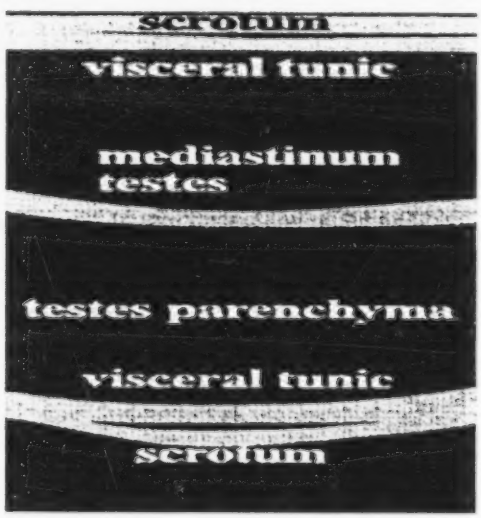
TIFF (LZW) decompressor
are needed to see this picture.



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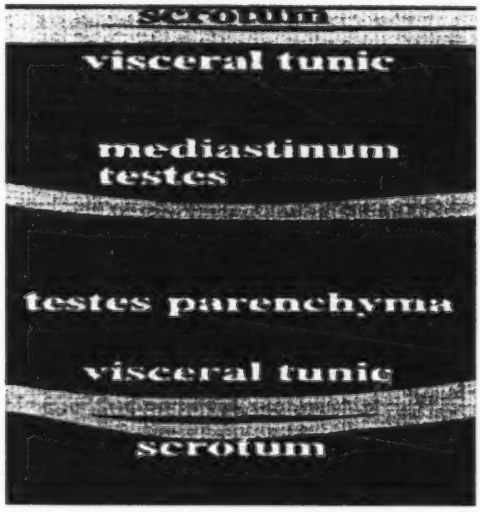


STUDIES OF TESTIS FIBROSIS

I DETERMINATION OF THE TIME OF ONSET OF TESTIS LESIONS AND THE PREVALENCE OF AFFECTED BULLS.

a. Bull calves raised at the University of Saskatchewan Goodale Farm

Calves were raised at pasture and weaned to a feedlot diet at approximately 7 mo of age. The calves were ultrasounded monthly for 12 months starting when they were 2-3 mo of age. None developed any testicular lesions.

b. Bull calves on a nutritional experiment at Lethbridge

Calves were weaned at 2 mo of age and raised from 2 - 15 mo on balanced rations containing 3 levels of energy and protein; 75, 100 and 125% of recommended intakes. Ultrasounds were done monthly from 3 - 15 months of age. At the end of the experiment, only 3 bulls in the low energy group had slight testicular fibrotic lesions.

c. Bulls at the Gunton ROP Test Station in Manitoba.

Ultrasound examinations of the testes of 185 bulls were done at the Gunton Test Station when bulls were approximately 8 months old and again when they were about 13 months old. At 8 mo of age 12.9% of bulls had testis lesions. Fibrotic lesions of the testes were classified as mild to moderate in 11.4% of bulls and severe in 1.5%. At approximately 12 mo of age, 26.5% of bulls were affected; 24.0% had mild to moderate fibrosis and 2.5% had severe fibrosis.

II ULTRASONOGRAPHIC EXAMINATIONS OF THE TESTES OF MATURE BULLS

a. Cases presented to the Large Animal Clinic at the WCVM

These bulls were studied early in this experiment and were primarily used to characterize the type and extent of lesions. Lesions detected included vesiculitis, orchitis, epididymitis, ampullitis, testicular fibrosis, testicular degeneration, and scrotal hernia.

b. AI Center bulls (Alta Genetics, Independent Breeders)

Of 54 bulls with semen problems, 52% had mild or moderate testis fibrosis. In those with fibrotic foci in the testes, semen quality was inadequate, but not different from problem-bulls without testis lesions. Thus persisting poor semen quality can occur without ultrasonically demonstrable lesions in the testis parenchyma.

c. University farm bulls in Cordoba Argentina

The proportion of bulls with testis lesions was much higher than expected. Of the 186 bulls ultrasounded in December, only 81 were free of testes lesions and 105 had fibrotic lesions in one or both testes (56.5%). In August, of 178 remaining bulls, 142 (79.8%) had lesions. This study appeared to indicate that nutritional management, i.e., pasture maintenance vs. rations for maximal growth, (Argentina vs. Manitoba ROP station) likely does not contribute to testis fibrosis. In the spring of 2004, we learned that a viral disease had swept through all of the bulls 6 - 8 weeks prior to our December visit. The Bovine Respiratory Syncytial Virus (BRSV) was confirmed in lung tissue of bulls that died by the Saskatchewan Prairie Diagnostic Laboratory.

Semen was obtained from all of the bulls at the time of ultrasound examinations in August. Bulls with ≥70% normal sperm were considered to have a satisfactory semen score. Table 2 shows the combined scores of both testes bull and the corresponding

number of bulls with satisfactory semen quality. Combined testis scores of 1-4, 5-6, and 7-10 were considered as mild, moderate and severe, respectively.

Table 2. Fibrosis scores and corresponding numbers and percent of bulls with satisfactory semen quality in 178 bulls aged 17-19 months in August 2004.

Combined testis fibrosis score	**Number of bulls**	**n Satisfactory semen (%)**
No lesions	36	33 (91.7%)
Mild (1-4)	100	86 (86.0%)
Moderate (5-6)	21	16 (76.2%)
Severe (7-10)	19	15 (78.9%)

There was a tendency for more bulls to have inadequate semen quality when the testes were moderately or severely affected by fibrosis. However, even bulls with severe fibrosis could have satisfactory semen quality with up to 94% morphologically normal sperm

In August of 2004 a second new crop of 184 young bulls was available for examination at a second University of Cordoba facility. These calves had been vaccinated at 4-6 months of age for BRSV as well as other common disease agents. The calves were approximately 5-7 months of age at the time of our examination. Only 21 of 184 (11.4%) of bulls had any sign of testis lesions. In every case there were only a few tiny foci of fibrosis. We hope to re-examine these bulls in 2005. The evidence from these groups of bulls suggests that BRSV may be a cause of testis fibrosis.

III. THE EFFECT OF TESTIS DEGENERATION INDUCED BY SCROTAL INSULATION ON ULTRASOUND CHARACTERISTICS OF TESTIS TISSUE.

Scrotal insulation was applied for 4 days and 10 days in 2 groups of 8 bulls. Ultrasonography was done weekly for 10 weeks. All bulls suffered a severe deterioration in semen quality. Recovery to normal semen quality occurred within 6 weeks in bulls that were insulated for 4 days and within 10 weeks in 7 of 8 bulls that were insulated for 10 days. There were no significant changes in pixel intensity of testis tissue during the period of study after scrotal insulation in any of the 16 bulls.
Scar tissue did not form in response to prolonged scrotal insulation. Therefore, it seems unlikely that cases of testis fibrosis encountered in the field are due to abnormal thermoregulation of the testes.

Discussion

The prevalence of fibrotic lesions in the testes

This study indicated that some populations of bulls in western Canada have a high incidence of bulls affected by fibrotic lesions in the testis. Other populations of bulls had no fibrotic lesions or a low incidence of lesions. It is likely that testis fibrosis occurs in many bull populations in many countries. Since there are large variations in the proportion of bulls affected in different herds in different locations it seems likely that differences in

the management of young bulls may be involved in the development of testis tissue disease

The cause of testis fibrosis

Tubularization of the seminiferous cords within the testis tissue is completed in nearly all bulls by 6 mo of age. Sperm release into the tubules occurs occurs in most bulls at about 10 mo of age. Therefore, tubule-impaction and rupture with escape of sperm into the peritubular space of the testes tissue, could result in a reaction to sperm and infiltration of scar tissue. The hypothesis of tubule impaction and rupture fits the fibrosis pattern we are seeing.

Bacterial and viral infections occur most commonly in the early postweaning period when maternal immunity has waned. Thus an infectious cause of fibrosis, would fit the time when lesions are appearing in young bulls. Bacterial infections would be expected to cause very visible swelling and pain of the testes and since this was not seen a bacterial cause seems unlikely.

Viral infections may target specific cells within the seminiferous tubules resulting in tubule damage and fibrous tissue infiltration. Indeed, fibrotic lesions similar to those we've described in bulls were reported recently in boars infected with the Circa virus. Our studies in Argentina suggest that the Bovine Respiratory Syncytial Virus (BRSV) may cause testis fibrosis. BRSV is a frequent cause of respiratory disease in weaned calves in Canada. Furthermore, western Canadian calves are less likely to be vaccininated for BRSV than for other viral diseases.

It seems unlikely that the level of nutritional intake is involved in the production of scar tissue since bulls on low, medium and high (excessive) intakes at the Lethbridge Research Station had a very low incidence of the problem and the level of intake was not associated with excessive nutritional intake.

The scrotal insulation experiment demonstrated that abnormal thermoregulation of the testes, such as in cases of obesity, is an unlikely cause of testis fibrosis. However, it is possible that in cases of very prolonged abnormal thermoregulation scar tissue infiltration might occur.

The significance of fibrotic lesions in the testes

A high proportion of bulls in AI centers in Canada with persistent inadequacy of semen quality (52%) had mild testis fibrosis. In the study done at Cordoba, there was a tendency for more bulls to have inadequate semen quality when the testes were moderately or severely affected by fibrosis. However, even bulls with severe fibrosis could have satisfactory semen quality with up to 94% morphologically normal sperm. Thus even large amounts of scar tissue within the testis parenchyma did not prevent the remaining unaffected parenchyma from producing normal sperm. One would expect a geatly reduced sperm output from testis with large amounts of scar tissue. This would likely result in reduced fertility in bulls with high numbers of cows to breed within a short time frame.

STUDIES OF PATHOLOGY OF THE CAUDA EPIDIDYMIDES

We found 5 cases of abnormal cauda epididymides during this study. We were able to study 3 of the cases. One of the cases of cauda epididymitis recovered spontaneously

after 3 months. Two bulls did not recover over a 6-month period. In the 2 bulls that did not recover, ultrasonography and post castration dissection showed that cauda enlargement was due to cystic cavities containing spermatozoa. It appeared that the most likely cause of the inflammation and sperm/fluid accumulation was rupture of the epididymal tube. In such cases it would be unlikely that a bull could re-establish sperm passage through the duct system.

Normal cauda epididymis

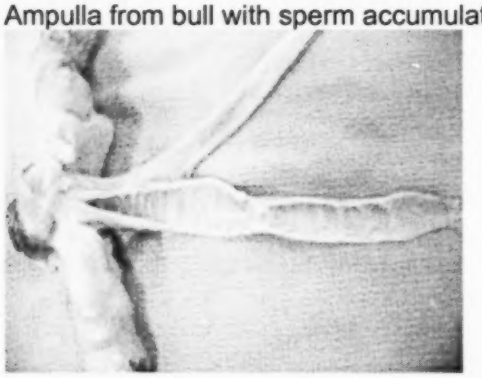
Normal cauda sonogram

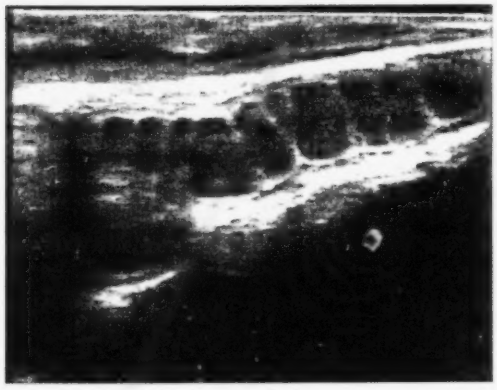
Spermiocoele in cauda

STUDIES OF AMPULLARY ACCUMULATION OF DEAD SPERM

The ampullae are glandular dilations of the vas deferens which transports sperm from the testes to the urethra. The ampullae are located near the junction of the vas deferens with the urethra. A function for the ampullae has only been vaguely described. For many years, we have known about the phenomenon accumulation of senescent sperm in bulls and stallions. The condition was associated with infertility and bulls were generally culled. Until our recent ultrasound studies we believed that the sperm accumulated in the cauda epididymis and that with frequent ejaculation, the senescent sperm might be flushed out of the system with a possible return to normal fertility. Our studies showed that dead sperm accumulated mainly in the ampullae and this is readily visualized with ultrasonography.

Ampulla from bull with sperm accumulation





It appears that in normal bulls, sperm move out of the cauda epididymis, through the ampullae and into the urethra. Sperm are, therefore, continuously lost into the urinary system. This ensures that fresh sperm are available at any time for ejaculation regardless of sexual rest.

Abnormal accumulation of senescence sperm in the ampullae results in ejaculates of mainly dead sperm. Breeding trials indicated that it takes about a week of breeding for bulls to rid themselves of senescent sperm and then pregnancies begin to occur a normal rate. The period of sexual rest required for re-accumulation of dead sperm is 4-6 weeks.

Technical Report

Introduction

During routine bull breeding soundness evaluation bulls with poor semen quality are frequently found. Veterinarians are often expected to provide a prognosis for recovery within a given time period based on one physical examination and semen evaluation. Ultrasonography has the potential to provide additional information about the health of reproductive tissues and should be a very useful adjunct to routine bull evaluation. There are very few reported studies on ultrasonography of the bull reproductive tract. A slaughter plant study to characterize the normal appearance of the testis and the potential application of ultrasonography to measure testis size has been previously reported. In addition, our colleagues at the WCVM reported on echogenic changes of testis tissue during pubertal development. To our knowledge, this is the first extensive ultrasonographic study on abnormalities of the bull reproductive tract.

The objective of this research is to characterize the normal and abnormal ultrasonographic features of reproductive tissues, to determine the prevalence of reproductive tract lesions in different age groups of bulls, to correlate ultrasonographic information with semen quality traits, and to use ultrasonography in conjunction with physical findings and semen information to formulate a prognosis for future bull fertility.

Equipment and Methodology

a. Recording and storing ultrasound images and image data.

Ultrasound images of tissues are essentially a map of pixels of different intensities. In the past, images produced by an ultrasound machine were recorded on video and then transferred to a computer for pixel analysis. Loss of data and distortion of data by this system made it difficult to produce reliable and statistically analyzable data. The need to carry a video recorder with an ultrasound machine and a desk-top computer often over large distances, into barns or corral environments further complicated this type of study. Therefore, feasibility studies were done to directly interface an ultrasound machine with a lap top computer. After a great deal of frustrating work with a number of different ultrasound machines and computers we found that recent models of the Aloka 900 ultrasound machines had a built in image analysis system allowing instantaneous data capture and pixel intensity analysis. Furthermore, the Aloka 900 could be interfaced to an Apple iBook with a Dazzle mage capturing device. This made travel with equipment, work in barn environments and reliable data management feasible. Image data was saved on the iBook laptop computer and on CDs.

b. Technique of ultrasonography and the establishing ultrasonographic features of normal tissues

Compression of tissues with the ultrasound transducer head alters the tissue density slightly and results in reflection of differing intensities of ultrasound and thus different intensities of pixel intensity from different measurements of the same tissue. Therefore, identical pressures must be used on tissue each time measurements are done. There have been reports in the literature from Europe of a pressure-measuring device that was coupled with the ultrasound transducer head. We contacted the author of this work and he came to our college and demonstrated his equipment. However, we found that the rather cumbersome equipment did not provide more reliable data than a simpler subjective method. In the subjective method, we used a gradual reduction in contact pressure with the transducer head until contact was almost lost. Then the image was frozen and recorded. We did a comparative repeatability study on pixel intensity of testis tissue of 10 normal bulls and found this subjective method to result in highly repeatable data.

c. Development of a testis tissue biopsy instrument

Initially we believed that it would be important to correlate ultrasonographic data with histological (tissue microscopic) data. The histological data would be determined from tissue biopsies. To our knowledge, no one has reported a successful method of testis biopsy in animals that does not involve general anesthesia and surgery. None of the needle biopsy instruments available on the market today perform satisfactorily. With the help of U of S engineers we developed a biopsy instrument that would consistently provide a useful tissue sample. The design involved hollowing out a 12-gauge needle behind a cutting edge to allow the cut tissue core an easy passage into the needle. We devised a serrated tip that cuts the tissue upon rotation of the needle. Using suction on the biopsy needle during rotation ensured coring a clean-cut sample. We did pre-slaughter biopsies on 4 bulls without causing hematomas, or significant hemorrhage. However, it soon became evident from our ultrasound studies that in most cases lesions are not uniformly distributed through out the testis, but rather occur in discrete units within the parenchyma. It is possible to obtain a biopsy of discrete lesions, however, not without a large degree of

difficulty and low potential usefulness of samples. Therefore, we decided early on not to pursue the use of biopsies in this study.

d. Reproductive tissues studied

Ultrasound transducers have been designed to function by direct introduction into the rectum or to be applied to the skin surface using a gel to provide good contact. The internal reproductive tract is accessible to ultrasound via transrectal palpation. The main internal structures of interest in bulls are the ampullae and vesicular glands, and externally, the testes and epididymides. We found a surprisingly high prevalence of bulls with fibrotic lesions in the testis and testis fibrosis became the major focus of this study. We did a small amount of work on vesiculitis and epididymitis.

We obtained ultrasound images of normal and inflamed vesicular glands, however, since ADF funding was obtained for a more intensive study of this problem, we did not concentrate on vesiculitis as part of this project. Briefly, in the other project focusing on vesiculitis, ultrasonographic data was obtained from 14 bulls with clinical vesicular gland infections, and included in the data were bacterial cultures, response to antibiotic treatment and post recovery ultrasonograms. A great deal of additional work on vesiculitis was done on that project that did not involve ultrasonography.

The cauda epididymides may be enlarged due to inflammation, traumatic rupture or it may missing due to hereditary segmental aplasia of the Wolfian duct system. However, the incidence of epididymal problems is very low. We were able to study 3 of 5 bulls with enlargement of the cauda epididymis, and some of the images are included in this report.

An earlier study done by the author showed that accumulation of dead sperm involving the ampullae occurs in about 1% of all bulls. Ultrasonography was used for the first time to demonstrate dilation of the ampullae in the phenomenon of sperm accumulation. A complete study of ampullary sperm accumulation was done and is reported here.

e. Bull populations studied

1. University of Saskatchewan Goodale Farm.

About 70 bull calves per year are born at this farm. Although there is a great deal of demand for use of these calves in different studies, we were able to obtain 41 calves to monitor by ultrasound from calf hood through to maturity.

2. Lethbridge Research Station.

Our group is collaborating with this research station on nutritional studies using bull calves weaned at about 2 months of age. We were able to ultrasonographically evaluate the effect of low, medium and high nutritional intakes on testis development from calf hood through to maturity.

3. Gunton ROP Test Station in Manitoba.

A large group of bulls were ultrasounded at about 8 months of age and again at about 13 months of age. This allowed the determination of the prevalence of tissue lesions in young bulls from a variety of farm backgrounds.

4. Community Pasture bulls and cases at the Western College of Veterinary Medicine (WCVM)

This group allowed us to examine range bulls at a variety of different ages.

5. AI Center bulls at Alta Genetics and Independent breeders.

These bulls were a good resource of problem bulls that that had continuous semen collection and evaluation data.

6. The Catholic University in Cordoba Argentina.

The WCVM has developed a collaborative relationship with this university in graduate programs and research. The Catholic University owns a large population of cattle and produces over 200 bulls per year for development of a new composite breed, for purposes of breed improvement, bulls for use in their AI center and bulls for general research and teaching. We were able to access this group to compare bulls raised by a somewhat more extensive rangeland system than bulls raised western Canada.

f. Quantifying testis fibrosis

One of the most difficult aspects of this research was to quantify the patterns, sizes, and frequency of fibrotic lesions within the testes. Initially, we thought this could be most logically accomplished by comparing the amount of fibrosis to semen quality. However, in light of our findings in the Argentine work this past year in which bulls with severe fibrosis could produce good semen quality, we constructed a purely arbitrary scoring system for testis fibrosis. We decided on a 6-point scale of fibrosis (scores of 0 - 5 per testis) in which 0 means a normal homogenous pattern of echotexture throughout the testis with no lesions and scores from 1 to 5 encompass slight fibrotic change to very severe fibrosis, respectively (Table 1 and Figures 1-6). The sum of the scores for both testes can be used to give a total score, thus very severe fibrosis in both testes would give a score of 10. Most sonograms of affected testes had a mixture of lesion sizes, however, some had only one category of lesion size. Usually both testes were affected equally or almost equally.

Definitions used in quantification are as follows:

Small foci of fibrosis: 1-2 mm wide and 1-3 mm long
Medium foci of fibrosis: 2-5 mm wide and 3-10 mm long
Large foci of fibrosis: >5mm wide and >10 mm long

Table 1. Classification method for testis fibrotic lesions.

	Number of fibrotic lesions		
Category	Small	Medium	Large
0. Normal	0	0	0
1. Slight	1-5	1-2	0
2. Mild	6-10	3-5	1-2
3. Moderate	11-20	6-10	3-5
4. Severe	21-30	11-15	6-10
5. Very Severe	>30	>15	>10

The category of Very Severe could also include obliterating fibrosis in which large areas of fibrosis block the ultrasound beam path resulting in broad non-echogenic areas

beyond the fibrous mass and involving >25% of the testis. Several bulls (<1%) had 3 -5 mm non-echoic (black) foci in one or several places within the parenchyma. In one bull this was shown by dissection to be due to enlarged blood vessels within the testis parenchyma. No significance has been attached to this finding at this time.

Results

STUDIES OF TESTIS FIBROSIS IN DIFFERENT GROUPS OF BULLS

I Ultrasonographic examinations of calves and young bulls to determine the time of onset of testis lesions and the prevalence of affected bulls.

Ultimately, we need to determine the cause of these lesions before we can learn how to prevent them. There may be more than one cause and there may be multiple factors involved within the cause. Fibrotic lesions in the testes may be due to Environmental causes which include infectious organisms, nutrition, trauma, and toxicity, or Congenital and Genetic causes. Environmental - genetic interactions are also possible. Some clues might be provided by determining at what age lesions develop, at what stage of testicular development they occur and under what kind of management conditions they occur most frequently. Therefore a large portion of the study was devoted to ultrasonography in calves and young bulls.

a. Bull calves raised at the University of Saskatchewan Goodale Farm

These calves (n = 41) were raised by the conventional method nursing their dams at pasture and weaned to a feedlot diet at approximately 7 mo of age. This was an opportunity to monitor testis development from a very young age in calves reared in the traditional manner in western Canada. Calves were vaccinated with an 8-way clostridial vaccine and a modified live IBR/BVD vaccine at weaning. Calves were weaned with the Quietwean nose tag system to reduce weaning stress. None of the calves developed respiratory disease after weaning. The calves were ultrasounded monthly for 12 months starting when they were 2-3 mo of age. None of the calves developed any testicular lesions.

b. Bulls on a nutritional experiment at Lethbridge

In another experiment, we worked with groups of bulls at the Agriculture Canada Lethbridge Research Station to determine the effect of different levels of nutrition in calf hood and after weaning on the pubertal development of beef bulls. The bulls were weaned at 2 mo of age and were raised from 2 mo to 15 mo on balanced rations containing 3 levels of energy and protein; 75, 100 and 125% of recommended intakes. Calves were vaccinated for the main viral and bacterial diseases. These bulls provided us with an opportunity to follow the development of testis lesions from their earliest stages as well as to determine whether nutritional intake influences their development. Two groups of bulls were ultrasounded, one in beginning in 2002 (n = 39), and one in 2003 (n = 44). Ultrasounds were done monthly from 3 - 15 months of age. At the end of the experiment, only 3 bulls had slight testicular fibrotic lesions. Interestingly, the 3 bulls were in the low energy group.

c. Bulls at the Gunton ROP Test Station in Manitoba.

Ultrasound examinations of the testes of 185 bulls were done at the Gunton Test Station when bulls were approximately 8 months of age and again when they were about 13 months of age. Pre-sale semen samples were taken at the end of the test period. At 8 mo of age 12.9% of bulls were affected. Fibrotic lesions of the testes were classified as mild to moderate in 11.4% of bulls and severe in 1.5%. At approximately 12 mo of age, 26.5% of bulls were affected; 24.0% had mild to moderate fibrosis and 2.5% had severe fibrosis. Only 16 of 49 bulls with testicular lesions had unsatisfactory semen quality at 12 - 14 mo of age. However, the poor semen quality in many or most of these bulls could be due to immaturity. Indeed, a similar proportion of bulls without testicular lesions had poor semen quality. Since these bulls are widely dispersed after the spring sale, further follow up on their semen quality was not possible.

This study demonstrated that fibrotic lesions appear in the testes of bulls as young as 8 months of age and as time goes on the incidence of affected bulls increases, but the severity of fibrosis does not increase appreciably. In western Canadian bull populations 10-15% are expected to suffer from inadequate semen quality. However, in this case, 26.5% of bulls had evidence of testis parenchymal damage. Therefore, it is likely that many bulls with fibrotic foci in the testes produce normal semen.

II Ultrasonographic examinations of the testes of mature bulls

In yearling bulls abnormal semen quality is often due to immaturity. Maturity is attained in approximately 33% and 60% of 12 month-old and 14 month-old bulls, respectively. At 16 months of age most bulls have attained maturity. Therefore, to correlate testis lesions with semen quality bulls must be mature.

a. Cases presented to the Large Animal Clinic at the WCVM

Ultrasonically detectable lesions of the reproductive tract were found 23 bulls from among 177 internal clinical cases and 449 community-pasture bulls. Lesions detected in these bulls included vesiculitis, orchitis, epididymitis, ampullitis, testicular fibrosis, testicular degeneration, and scrotal hernia. These data do not represent a prevalence rate for fibrosis in this group of bulls since ultrasound was done primarily on bulls with indications of problems through the physical exam and examination of semen samples. These bulls were studied early in this experiment and were primarily used to characterize the type and extent of lesions.

Three bulls with enlarged cauda epididymides were monitored for 6 mo. One bull recovered spontaneously and 2 showed no improvement in the cauda epididymides during this time period. The 2 bulls were castrated and their testes and epididymides were recovered and examined. Bacterial culture results from epididymal material were negative. In both cases epididymal enlargement was due to spermatocoeles (walled-off accumulations of sperm). It appeared that the cause of the cauda enlargement was trauma leading to rupture of the epididymal duct and accumulation of sperm. Biopsies from both testes in those 2 bulls were done and their testis tissue samples were found to be histologically normal.

b. AI Center bulls (Alta Genetics, Independent Breeders)

These bulls provided a unique opportunity for studying the usefulness of ultrasonography since a detailed semen production record was available on each bull. A total of 61 bulls were examined by ultrasound. Of these, 54 bulls had persisting problems with inadequate semen quality for AI purposes and 7 bulls were arbitrarily chosen for good semen quality as controls. The latter 7 bulls had normal sonograms.

Of the 54 bulls with semen problems, 28 (52%) had mild or moderate testis fibrosis. One bull also had testis hypoplasia, and one had tumor-like masses in the testis parenchyma identified by ultrasonography. In the group with fibrotic foci in the testes, semen quality was inadequate, but as in bulls without lesions, semen changes were not severe. This study showed that persisting poor semen quality may occur without ultrasonically demonstrable lesions in the testis parenchyma. Therefore, testis lesions were not necessarily linked to inadequate semen quality.

The testes from one bull with persistent poor semen quality, but normal ultrasonograms, were recovered at slaughter for histological study. The microscopic analysis revealed testis tubular degeneration and mild fibrosis. Therefore, ultrasonography did not reveal degenerative changes that could be seen at the microscopic level.

c. University farm bulls in Cordoba Argentina

Due to many years of collaboration with colleagues at this University, we were able to obtain access to a large group of bulls for ultrasonography and semen collection. This group of bulls was advantageous for study since they were raised from calf hood through maturity and maintained at one location.

In December 2003, a trip was made to the veterinary college at the Catholic University of Cordoba where 186 bulls aged about 9-11 months were available for testicular ultrasonography. These bulls, were primarily of Angus and Hereford breeds and are not very different genetically from North American Hereford and Angus cattle since a great deal of Canadian and American semen has been imported into Argentina for many years. The bulls are somewhat unique to this study since they were raised to maturity on pasture in a foreign country. Under their system of management, bulls grow somewhat slower and mature about 2 months later than bulls raised in western Canada. Therefore, the rate of testes development in Argentine bulls is somewhat slower than bulls raised in western Canada. At about 16 months of age the bulls were placed in a feedlot and fed grains and hay to fit them for sale as 2-yr-old bulls. In August of 2004 when the bulls were approximately 18-20 months of age the bulls were ultrasounded again and semen was collected by electroejaculation. In August, 178 of the original bulls were available.

The first results from our investigation of bulls in Argentina were unexpected. To our surprise the proportion of bulls affected with testis lesions was much higher than we have experienced in western Canada. Of the 186 bulls ultrasounded in December, only 81 were free of testes lesions and 105 had fibrotic lesions in one or both testes (56.5%). In August, of 178 remaining bulls, only 36 bulls remained entirely free of testis lesions; 142 of 178 (79.8%) had lesions (Tables 2 and 3). This study appeared to indicate that nutritional management, i.e., pasture maintenance vs. rations for maximal growth, (Argentina vs. Manitoba ROP station) likely does not contribute to testis fibrosis, unless slower testis development on pasture is detrimental.

In the spring of 2004, it came to our attention that a viral disease had swept through all of the bulls 6 - 8 weeks prior to our December visit. Most of the bulls showed signs of fever or respiratory disease and some of the bulls died. At necropsy of bulls that died, one of our former graduate students recognized lung lesions to be indicative of Bovine Respiratory Syncytial Virus (BRSV). Lung tissues that had been preserved were sent to the Prairie Diagnostic Laboratory in Saskatoon. The respiratory disease was confirmed by immunofluorescent studies to be incited by the BRSV virus. In August of 2004, 10 bulls that were of insufficient quality for sale were sent to slaughter in Cordoba and testis tissues were recovered. However, none of these ten bulls had fibrotic lesions in the testes. Nevertheless, tissue samples were preserved in formalin and brought back to the Prairie Diagostic center in Saskatoon to assay the tissues for BRSV fluorescent antibodies. At this time, the laboratory has not yet reported the results.

Table 2. Testis fibrosis scores in 186 bulls aged 9 - 11 months in December 2003.

Fibrosis Score	Right testis (n)	Left Testis (n)
0 (no lesions)	81	110
1-2 (slight - mild)	81	54
3 (moderate)	15	13
4-5 (severe - very severe)	9	9

Table 3. Testis fibrosis scores in 178 bulls aged 17 - 19 months in August 2003.

Fibrosis Score	Right testis (n)	Left Testis (n)
0 (no lesions)	60	59
1-2 (slight - mild)	84	82
3 (moderate)	15	26
4-5 (severe - very severe)	19	11

Semen was obtained from all of the bulls at the time of ultrasound examinations in August. Bulls with ≥70% normal sperm were considered to have a satisfactory semen score. Table 4 shows the combined scores of both testes bull and the corresponding number of bulls with satisfactory semen quality. Combined testis scores of 1-4, 5-6, and 7-10 were considered as mild, moderate and severe, respectively.

Table 4. Fibrosis scores and corresponding numbers and percentages of bulls with satisfactory semen quality in 178 bulls aged 17 - 19 months in August 2004.

Combined testis fibrosis score	Number of bulls	n Satisfactory semen (%)

No lesions	36	33 (91.7%)
Mild (1-4)	100	86 (86.0%)
Moderate (5-6)	21	16 (76.2%)
Severe (7-10)	19	15 (78.9%)

These bulls were mature and had been in a feedlot and free of infectious diseases for over 4 months, therefore, the correlations of semen quality with testis lesions were not confounded by the effects of illness or immaturity. There was a tendency for more bulls to have inadequate semen quality when the testes were moderately or severely affected by fibrosis. However, even bulls with severe fibrosis could have satisfactory semen quality with up to 94% morphologically normal sperm. These results indicate that the presence of even relatively large amounts of scar tissue within the testis parenchyma did not prevent the remaining unaffected parenchyma from producing normal sperm. One would expect a reduced sperm output from testis with large amounts of scar tissue. The reduced sperm output might result in reduced fertility in bulls with high numbers of cows to breed within a short time frame. However, the amount of semen produced by bulls with different degrees of severity of fibrosis has not been investigated.

In August of 2004 a second new crop of 184 young bulls was available for ultrasound examination at a second University of Cordoba facility. These calves had been vaccinated at 4-6 months of age for BRSV as well as other common disease agents. The calves were approximately 5-7 months of age at the time of our examination. Only 21 of 184 (11.4%) of bulls had any sign of testis lesions. In every case there were only a few tiny foci of fibrosis. We hope to re-examine these bulls in 2005. If this second group remains relatively free of testis fibrosis, the evidence would point to BRSV as a cause of fibrotic lesions in the testes of bulls.

III. Scrotal Insulation to determine whether abnormal testis thermoregulation might be cause of testis fibrosis

We speculated that some of the potential causes of testis fibrosis, were abnormal thermoregulation of the testes (heat), trauma, bacterial infection, viral infection or a tissue reaction to sperm that escaped from impacted seminiferous tubules.

Our earlier work, and the work of others, has shown that abnormal thermoregulation (heating) of the testes by as little as 1 ^{0}C will cause testis degeneration. This degeneration is manifested as death and sloughing of the germinal cells lining the seminiferous (sperm producing) tubules that make up the testis tissue. Our earlier work showed that 4 or 7 days of scrotal insulation, or 7 days of testosterone suppression by simulated stress, resulted in degeneration followed by regeneration in 6 to 8 weeks. Semen quality and total sperm output was severely impaired but returned to normal after seminiferous tubule regeneration. In our clinical experiences we have found that in most naturally occurring cases of testicular degeneration, such as those caused by prolonged stress or obesity, regeneration is possible after the cause of abnormal spermatogenesis is removed.

We postulated that with severe and prolonged abnormal thermoregulation, recovery might not occur and scar tissue infiltration might occur. With this in mind we designed an experiment to determine whether one of the causes of testis fibrosis is abnormal thermoregulation of the testes.

Hypothesis:

a. Mild degeneration (sloughed germinal cells in the seminiferous tubules) could be detected by ultrasonographic measurement of the pixel intensity of testis tissue.

b. Prolonged insulation would result in some irreversible damage to testis tissue resulting in fibrous tissue infiltration that would be detectable by ultrasonography.

Methods

Sixteen bulls with normal, well-developed testes and normal semen quality were used in the experiment. 8 bulls had scrotal insulation for 4 days and 8 bulls had scrotal insulation for 10 days. The testes were ultrasounded weekly for until recovery to normal semen quality in all bulls and then monthly for an additional 3 months in one bull that did not recover. Semen was collected and evaluated at the same time as ultrasonography was performed.

Results

All bulls suffered a severe deterioration in semen quality. Recovery to normal semen quality occurred within 6 weeks in all bulls that were insulated for 4 days. Recovery to normal semen quality occurred within 10 weeks in 7 of 8 bulls that were insulated for 10 days. In one bull, semen quality remained mildly inadequate for 5 months and then the bull was castrated. There were no significant changes in pixel intensity of testis tissue during the period of study after scrotal insulation in any of the 16 bulls. Several permanent hypoechogenic areas appeared in the testis parenchyma within 3 weeks of insulation in the 10-day insulation bull that did not recover his semen quality. Upon castration and testes dissection it was determined that the hypoechogenic areas were enlarged arteries within the parenchyma of the testes. This is a surprising response to insulation that has not been described previously.

Conclusions

Scar tissue did not form in response even to prolonged scrotal insulation. Therefore, it seems unlikely that cases of testis fibrosis encountered in the field are due to abnormal thermoregulation of the testes, e.g., bulls with excessive fat deposition in the neck of the scrotum. Although we did not do an experiment to simulate stress, previous studies by the author which compared the effects of stress and heat on testis degeneration and regeneration indicated that short term stresses, such as those due to temporary cold and starvation, or severe foot rot, do not result in the deposition of fibrous tissue in the testis parenchyma. However, it is possible that severe prolonged heating of the testes or prolonged stress could cause such severe degeneration that scar tissue infiltration would occur. To our knowledge, this hypothesis has not been studied or reported.

This study concluded that pixel intensity analysis of testis tissue is unlikely to be a useful tool to study testes degeneration in bulls and will not be useful to practicing veterinarians for making a prognosis for recovery from poor semen production.

Discussion

The prevalence of fibrotic lesions in the testes

This study indicated that some populations of bulls in western Canada have a high incidence of bulls affected by fibrotic lesions in the testis. For example 26.5% of bulls in the Gunton ROP Test Station were affected. Other populations of bulls had no fibrotic lesions or a low incidence of lesions. A high percentage of bulls in a university herd in Argentina were affected. Therefore, it is likely that testis fibrosis occurs in many bull populations in many countries. Since there are large variations in the proportion of bulls affected in different herds and in different locations, it seems likely that differences in the management of young bulls may be involved in the development of testis tissue disease. Differences in disease exposure, vaccination practices, nutritional programs, housing, and possibly genetic backgrounds could influence the development of testis lesions

The cause of testis fibrosis

Tubularization of the seminiferous cords within the testis tissue begins at about 4 mo of age and is completed in nearly all bulls by 6 mo of age. Sperm release into the tubules occurs as early as 8 mo of age and occurs in most bulls by 10 mo of age. Therefore, tubule-impaction and rupture with escape of sperm into the peritubular space of the testis tissue, would not be expected to happen much before 10 mo of age. Since sperm are antigenically foreign, a tissue reaction against escaped sperm could result in fibrosis. Impaction of the tubules might be due to a congenital failure to attach to the rete tubules (a central column of tubules in the testes connecting the seminiferous tubules to the epididymis). With very rapid testis development tubularization of some of the seminiferous cords may be incomplete before sperm production begins. In particular, a delay in tubularization at the seminiferous tubule connection to the rete testis might be expected since this junction acts as a backflow valve within the normal tubular system. If the seminiferous duct system lacked patency in some areas, newly produced sperm would have no place to go and impaction could develop. Tubule rupture and a tissue reaction against sperm should not occur until the testes are capable of producing sperm. As sperm production increases toward the time of sexual maturity, testes lesions would be expected to increase. The hypothesis of tubule impaction and rupture does fit to some degree the pattern we are seeing. For example, the first lesions are showing up at about 8 mo of age and by the time bulls are 12-14 mo old and many have matured, or are reaching maturity, the percentage of bulls with fibrotic lesions increases. The data from the Goodale farm, Lethbridge research station and Gunton test station seem to indicate that bulls may show evidence of a disease process in the testes as early as 8 mo of age and that additional cases develop during pubertal development between 8 and 12 mo of age. The severity of lesions did not appear to change much between first detection at 8 mo and a second examination at 12 mo of age; therefore, the process that causes fibrosis does not seem to be progressive. The majority of lesions seen in young bulls in this experiment appear to involve lobules or discrete tubules within the parenchyma. This observation suggests individual tubule damage and partial or complete tubule disintegration in the development of fibrotic lesions. Maldevelopment of the tubule-to-rete connection due to very rapid testis

growth, a genetic or congenital defect of tubule-rete testis development, or a viral disease process affecting tubule health and development are possibilities in the etiology of tubule destruction and fibrosis.

Bacterial and viral infections occur most commonly in the early postweaning period when maternal immunity has waned and animals from different backgrounds are mixed and held together in close quarters. Thus an infectious process as a cause of fibrotic lesions would fit the time period when testis lesions are appearing in young bulls. Bacterial infections would be expected to cause very visible swelling and pain of the testes, however, it is possible that some mild infections would be unnoticed. Nevertheless, if bacterial infections were a common cause of fibrotic testicular lesions, one would expect a much higher incidence of the very visible inflammation than has been seen in practice. Therefore, it seems unlikely that bacterial infections would account for the large number of cases of testis fibrosis.

Viral infection could cause inflammation of the arterioles and capillaries in the testes leading to local tissue necrosis and the formation of foci of fibrosis. Viral infections may also target specific cells within the seminiferous tubules such as the Sertoli cells or individual germinal cells resulting in tubule damage and fibrous tissue infiltration. Indeed, fibrotic lesions similar to those we've seen in many of the cases in this experiment have been described recently in boars after infection with the Circa virus. Therefore, a viral cause remains a possibility. The two most common viral diseases of cattle, bovine herpes virus (IBR) and bovine virus diarrhea virus (BVD) have been investigated for their role in male reproductive infections and have been found not to cause fibrotic lesions. However, other viruses need to be investigated. The investigation that took place in Argentina suggests that the Bovine Respiratory Syncytial virus may cause testis fibrosis. BRSV is a frequent cause of respiratory disease in weaned calves in Canada. Furthermore, western Canadian calves are less likely to be vaccininated for BRSV than for other viral diseases such as IBR and BVD.

It seems unlikely that the level of nutritional intake is involved in the production of scar tissue since bulls on low, medium and high (excessive) intakes at the Lethbridge Research Station had a very low incidence of the problem and the level of intake was not associated with excessive nutritional intake. Bulls at the Goodale farm and the Lethbridge station were vaccinated against the common viral ad bacterial diseases of cattle. This may have been the case at the Gunton Station as well, however in that case, vaccination was not uniformly done as bulls came from many different locations and had many different owners.

The scrotal insulation experiment demonstrated quite conclusively that abnormal thermoregulation of the testes, such as in cases of obesity, is an unlikely cause of testis fibrosis. In cases of prolonged abnormal thermoregulation, especially in cases where semen quantity and quality are very severely reduced, with loss of testis tone and size, and with severe seminiferous tubular degeneration, scar tissue infiltration might occur and result in testis fibrosis.

It is well known that in aged bulls, broad areas of fibrosis typically occur ventrally in the testes and it is speculated that the base of the testes is more susceptible to trauma. Trauma, caused by a blow to the testes from kicking or bunting could occur at any age, but might be more frequent during crowding in pens or frequent handling in chute systems. Therefore, the appearance of fibrosis in conjunction with post weaning penning and

feeding suggests trauma as a possible cause. However, one observation of the authors suggests that a few instances of trauma are unlikely to be the cause of the type of fibrotic lesions seen in this project. A bull was observed to receive a sharp blow to the testes from a metal bar. The bull's testes were ultrasounded the following day and several times over the course of a month later. In this case there was no ultrasonographic evidence of any kind of damage at all to the scrotum or testes.

The significance of fibrotic lesions in the testes

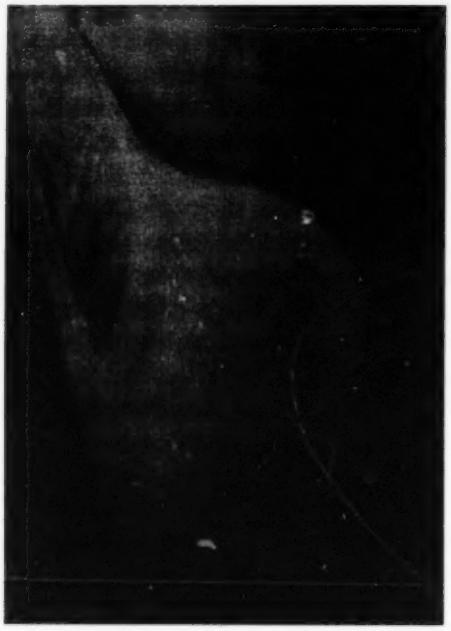
A high proportion of bulls in AI centers in Canada that had a persistent inadequacy of semen quality (52%) had some degree of testis fibrosis. This high percentage would seem to indicate an association between testis fibrosis and inadequate semen quality. In the study done at Cordoba, there was a tendency for more bulls to have inadequate semen quality when the testes were moderately or severely affected by fibrosis. However, even bulls with severe fibrosis could have satisfactory semen quality with up to 94% morphologically normal sperm. These results indicate that the presence of relatively large amounts of scar tissue within the testis parenchyma did not prevent the remaining unaffected parenchyma from producing normal sperm. Therefore, in cases with persisting abnormal semen quality and fibrotic lesions, the scar tissue itself likely is not the cause of sperm abnormalities, but rather an ongoing process separate from fibrosis, or perhaps resulting resulting in fibrosis, is at fault.

One would expect a reduced sperm output from testis with large amounts of scar tissue. The reduced sperm output might result in reduced fertility in bulls with large numbers of cows to breed within a short time frame. However, the amount of semen produced by bulls with different degrees of severity of fibrosis has not been investigated.

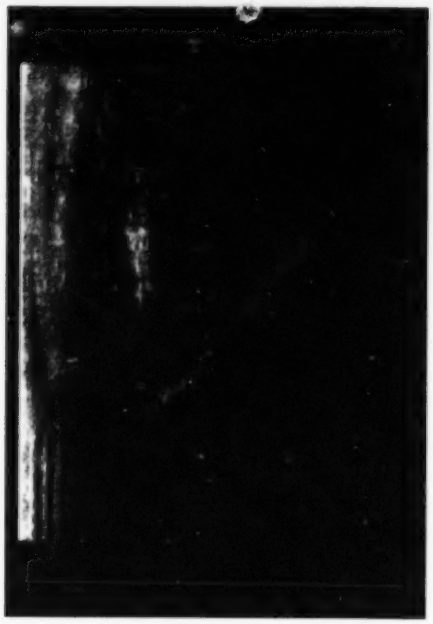
STUDIES OF PATHOLOGY OF THE CAUDA EPIDIDYMIDES

We found 5 cases of abnormal cauda epididymides during this study. We were able to study 3 of the cases. One of the cases of cauda epididymitis recovered spontaneously after 3 months. Two bulls did not recover over a 6-month period. In the 2 bulls that did not recover, ultrasonography and post castration dissection showed that cauda enlargement was due to cystic cavities containing spermatozoa. It appeared that the most likely cause of the inflammation and an accumulation of sperm and fluid was rupture of the epididymal tube. It is unknown whether a bull could re-establish sperm passage through the duct system after such a rupture.

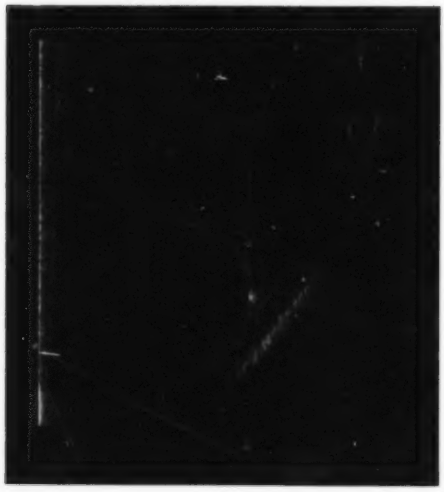
Normal cauda epididymis



Normal cauda sonogram



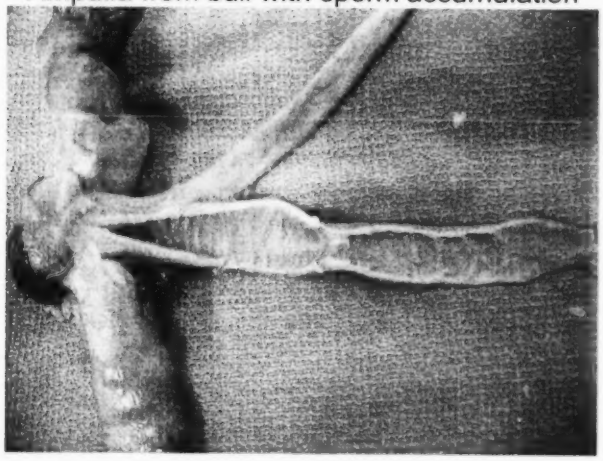
Spermiocoele in injured cauda



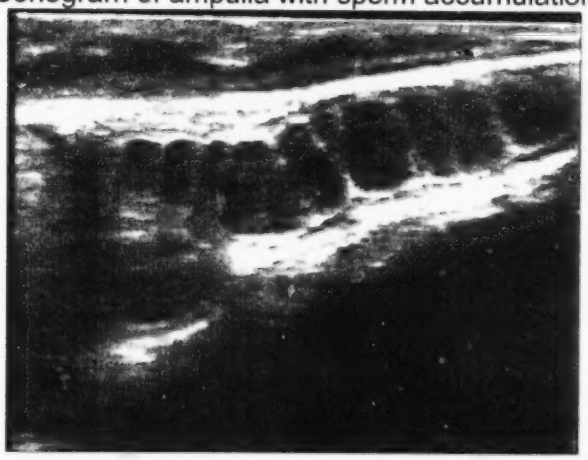
STUDIES OF AMPULLARY ACCUMULATION OF DEAD SPERM

During the early phase of this study 5 bulls with ampullary accumulation of sperm were discovered and used in this project. The ampullae are glandular dilations of the vas deferens which transports sperm from the testes to the urethra. The ampullae are located near the junction of the vas deferens with the urethra. A function for the ampullae has only been vaguely described. For many years, we have known about the phenomenon accumulation of senescent sperm in bulls and stallions. The condition was associated with infertility and bulls were generally culled. Until our recent ultrasound studies we believed that the sperm accumulated in the cauda epididymis and that with frequent ejaculation, the senescent sperm might be flushed out of the system with a possible return to normal fertility. Our studies showed that dead sperm accumulated mainly in the ampullae and this is readily visualized with ultrasonography. The cauda epididymis also has increased numbers of sperm in this disease.

Ampulla from bull with sperm accumulation



Sonogram of ampulla with sperm accumulation



It appears that in normal bulls, sperm move out of the cauda epididymis through the ampullae and into the urethra. Sperm are, therefore, continuously lost into the urinary system. This ensures that only fresh sperm are stored (as in an overflowing tank) and are fresh sperm are available at any time for ejaculation regardless of the period of sexual rest. Abnormal accumulation of sperm in the ampullae results in ejaculates with mostly dead sperm. We have now completely described the condition with ultrasonography. We have determined the number of ejaculations required to remove dead sperm whether by semen collection by artificial vagina, during natural service, or by electroejaculation. We have demonstrated through breeding trials that there is a return to normal fertility after dead sperm are flushed out of the system and we have investigated the period of sexual rest required (4-6 weeks) for re-accumulation of dead sperm in the ampullae. Some of the results of this work are shown below.

A. Results of depleting dead sperm from the ampullae through frequent ejaculation

Table 5. Spermiograms of 3 sperm accumulator bulls. Natural services 3 times per day per bull, using internal artificial vagina for semen collection. (Mean values per collection per day).

Day	1	2	3	4	5	6	7
Horned Hereford							
% Detached sperm heads	62	36	17				
% Sperm Alive	5	21	68				
% Normal Sperm	27	54	73				
Polled Hereford							
% Detached sperm heads	66	59	37	39	21		
% Sperm Alive	5	24	40	38	63		
% Normal Sperm	32	39	58	55	76		
Charolais							
% Detached sperm heads	68	52	42	41	32	19	18
% Sperm Alive	18	32	45	45	70	71	68
% Normal Sperm	28	38	49	56	67	67	76

B. Breeding trials using bulls with sperm accumulation

Two controlled breeding trials were done using 30 feedlot heifers per bull for 21 days of breeding to determine the effect of this condition on first service pregnancy rates. The bulls used were a 2-year-old Charolais bull and a 4-year-old Polled Hereford bull.

Breeding was observed for at least 2 hours twice daily and chin ball markers were also employed. Pregnancy was determined by ultrasonography 27 days after the end of the breeding period. From these observations it was possible to determine the order in which heifers were bred and whether pregnancy occurred.

Table 6. Pregnancy rates for 2 sperm accumulator bulls vs. normal control bulls.

	Charolais	Control bull
Week 1	2/12 = 16.7%	5/8 = 62.5%
Week 2	6/8 = 75%	6/6 = 100%
Week 3	9/10 = 90%	9/12 = 75%
21 day Pregnancy rate	17/30 = 56.7%	20/26 = 76.9%

	Polled Hereford	Control bull
Week 1	0/6 = 0%	3/5 = 60%
Week 2	6/8 = 75%	16/20 = 80%
Week 3	9/10 = 90%	2/4 = 50%
21 day Pregnancy rate	15/24 = 62.5%	21/29 = 72.4%

These results indicate that it takes about a week of breeding for bulls to rid themselves of senescent sperm and then pregnancies begin to occur a normal rate. The numbers of dead sperm and the location of sperm accumulation within the reproductive tract were determined after slaughter of sperm accumulator bulls. As expected most of the dead sperm accumulate in the ampullae, however, we showed that the cauda epididymis is also a site of sperm accumulation.

Table 7. Numbers of sperm found in the cauda epididymides of 2 normal bulls and 3 sperm accumulator bulls

Normal bulls	Sperm accumulator bulls
6.85×10^9	23.39×10^9
7.37×10^9	37.30×10^9
	15.74×10^9

Dissemination of Information (The ADF is credited for project support in each case)

a. Two conference presentations have been given thus far: The Western Canadian Association of Bovine practitioners, and the Institute of Animal Reproduction in Cordoba Argentina.

b. A paper entitled " Sperm accumulation in the ampulla and cauda epididymides in bulls" has been accepted for publication in Animal Reproduction Science.

c. A paper entitled "Relationship between semen quality and pixel intensity of testicular images after scrotal insulation in beef bulls" has been accepted for publication in Theriogenology.

d. After further analysis of accumulated a paper will be prepared for publication on the incidence and relationships of testis fibrosis to fertility.

e. An invitation for a presentation on this topic has been accepted for the joint American Veterinary Medical Association annual meeting and the World Veterinary Congress in Minnesota next July.